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The effect of antioxidants on the morphology and function of ovaries in rats inflammatory process (pregnancy) within the framework of the analysis of the assessment of their action and effectiveness on nuclear and cytoplasmic maturation of oocytes

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Abstract. The study aimed to investigate the effect of various antioxidants on the morphological and functional characteristics of ovaries in pregnant Wistar rats under conditions of systemic inflammation. The experimental research was conducted on a total of 20 sexually mature female rats of the Wistar strain, which were divided into five subgroups with four animals in each. The rats were categorised into the following groups: a control group (no treatment), an inflammation-induced group (lipopolysaccharide treatment), and three antioxidant-treated groups, where systemic inflammation was induced and the females were treated with resveratrol, quercetin, or lycopene. Systemic inflammation was induced using lipopolysaccharide, and different antioxidants were administered to the treated subgroups over a specified period from May to June 2023. The research focused on examining the impact of these antioxidants on oocyte morphology, mitochondrial function, and the levels of reactive oxygen species and glutathione. Results indicated that antioxidant treatment significantly improved oocyte morphology by reducing abnormalities and restoring normal mitochondrial distribution. Rats in the antioxidant-treated group exhibited reduced levels of reactive oxygen species and elevated concentrations of glutathione, signalling a reduction in oxidative stress. Additionally, an increase in the expression of genes associated with antioxidant defence was observed, suggesting an enhanced antioxidant response. These findings imply that antioxidants can improve oocyte quality in cases of inflammation-induced oxidative stress, offering new insights for reproductive biology. The practical relevance of this work lies in its potential application in reproductive medicine, particularly for patients with inflammation-related infertility, where antioxidant therapies could contribute to improving oocyte quality and reproductive outcomes

Keywords: post-ovulatory ageing; egg; antioxidant; oxidative imbalance; citrus coumarin

INTRODUCTION

Oxidative stress is a critical factor in various physiological and pathological processes, playing a significant role in the development of reproductive disorders. The ovaries, due to their high metabolic activity and sensitivity to oxidative damage, are particularly vulnerable to oxidative stress, especially during pregnancy. This vulnerability is heightened by hormonal changes and increased bodily demands during pregnancy, which can lead to alterations in ovarian function, folliculogenesis, and the overall success of pregnancy. Over the past few years, several studies have highlighted the damaging effects of oxidative stress on ovarian tissues and its potential role in infertility and other reproductive dysfunctions.

According to B. Bibak *et al*. [1], oxidative stress in the ovaries can lead to a reduction in oocyte quality, impair follicular development, and negatively affect hormonal balance, particularly the synthesis of progesterone, which is essential for supporting pregnancy. These findings emphasise the importance of managing oxidative stress in reproductive medicine, particularly in patients facing challenges

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during pregnancy or those with infertility issues. Similarly, study by V.R. Askari *et al*. [2] found that the presence of high levels of reactive oxygen species in the ovaries is associated with decreased fertility and lower implantation rates, suggesting that antioxidants may provide therapeutic potential in managing oxidative stress and improving reproductive outcomes.

The therapeutic use of antioxidants to combat oxidative stress in the reproductive system has been a growing area of interest. Research by V.R. Askari *et al*. [3] demonstrated that antioxidants such as resveratrol and quercetin significantly reduce reactive oxygen species levels in ovarian tissues, which in turn enhances ovarian function and improves oocyte quality. Furthermore, a study by S. Fiorito *et al*. [4] noted that lycopene and coenzyme Q10 (ubiquinone) may contribute to the stabilisation of the ovarian environment by mitigating the damaging effects of oxidative stress, suggesting that these antioxidants could be useful for improving fertility outcomes in women with oxidative stress-induced reproductive issues.

Despite promising results, a need for more detailed investigations into optimal antioxidant dosages, methods of administration, and long-term effects on ovarian function during pregnancy remains. Z. Keshavarzi *et al*. [5] examined the neuroprotective effects of auraptene following traumatic brain injury in male rats, focusing on the role of oxidative stress. Their findings demonstrated that auraptene significantly reduced oxidative stress markers and promoted cellular protection, highlighting its potential as a therapeutic agent under conditions of oxidative damage. However, their study did not explore its effects on reproductive functions, leaving room for further investigation into whether similar protective effects might apply to ovarian tissue under inflammatory conditions. Z. Tayarani-Najaran *et al*. [6] reviewed auraptene's properties as an anticancer agent, summarising its potential to inhibit tumor growth and reduce oxidative stress across various cancer models. While this review underscored auraptene's efficacy as a single-agent therapy, it also identified a lack of studies on the combined use of multiple antioxidants, which could potentially enhance protective benefits. These sources highlight the need for additional research on the combined effects of antioxidants, tailored dosages, and administration techniques, specifically in reproductive contexts where antioxidant therapy could be applied in clinical settings to improve ovarian function and pregnancy outcomes.

T. Vakili *et al*. [7] conducted a safety evaluation of auraptene, demonstrating that it exhibited low toxicity in both acute and subacute studies on rats, thus supporting its potential use in longer-term treatments for various health conditions. M. Igase *et al*. [8] explored the effects of auraptene from Citrus Kawachiensis peels on cognitive function in a randomised, placebo-controlled study in healthy volunteers, concluding that auraptene contributed positively to cognitive preservation, further indicating its antioxidant properties could benefit health beyond conventional applications. S. Galluzzi *et al*. [9] examined the cognitive and biological effects of citrus phytochemicals in individuals with subjective cognitive decline, showing that these compounds, rich in antioxidants, provided protective effects against cognitive deterioration over a 36-week period. Together, these studies highlight auraptene's efficacy in reducing oxidative damage and supporting cellular health, suggesting a broader application of antioxidants in physiological systems. Building upon these findings, this study aimed to evaluate the effects of a combination of antioxidants, including resveratrol, quercetin, lycopene, and ubiquinone, on oxidative stress in the ovaries of mature Wistar rats during pregnancy. This research examined effects on ovarian morphology, folliculogenesis, progesterone levels, and overall reproductive function, contributing valuable insights into the therapeutic potential of antioxidants for reducing oxidative stress and improving reproductive outcomes during pregnancy, thus expanding the possibilities for antioxidant use in preventing and treating oxidative stress-related reproductive disorders.

LITERATURE REVIEW

Oxidative stress significantly affects egg quality and overall female reproductive health, particularly in polycystic ovary syndrome, and M. Abizadeh *et al*. [10] are investigating the potential of auraptene to improve egg maturation, increase fertilisation rates, and reduce inflammation in a mouse model of polycystic ovary syndrome. Their findings indicate the potential use of auraptene as a therapeutic agent to improve reproductive function. A study by Y. Jang *et al*. [11] analysed the effects of auraptene on behavioural aspects in an experimental model of Parkinson's disease and its ability to protect mitochondrial respiration from inhibition and reduce reactive oxygen species The focus was on the ability to protect against inhibition of mitochondrial respiration and to reduce reactive oxygen species. This opens up the possibility of using auraptens not only in neurological diseases, but also in the field of reproductive medicine, as mitochondrial stress significantly affects the quality of oocytes. M.J. Lee *et al*. [12] found that auraptens promote the activation of antioxidant enzymes and improve brain endothelial cell connectivity by mitochondrial stress and enhance cellular function. This may be an important mechanism for improving oocyte maturation and functionality during aging; E.H. Hassanein *et al*. [13] have studied the role of coumarins such as auraptens in the regulation of the Keap1/Nrf2/ARE pathway, stimulating cellular antioxidant defences and positively affecting oocyte quality The study by N. Akino *et al*. [14] highlighted the possibility, focusing on the Sirt1/Nrf2 signalling pathway, which is involved in oocyte aging through the regulation of cyclin B1. These results highlight the importance of molecular interactions in maintaining reproductive health during the aging process.

Oxidative stress is an important topic in reproductive medicine research because of its significant impact on germ cell quality. V. Di Nisio *et al*. [15] investigated how oocyte aging after ovulation impairs oocyte quality and emphasise the importance of timeliness in reproductive technology. They pointed out that oocyte aging, both *in vivo* and *in vitro,* can negatively affect fertilisation success and embryo development. C. Yu & J.H. Xiao's [16] work focused on the Keap1-Nrf2 system, which mediates oxidative stress and aging. They emphasised that this system may be a target for therapies aimed at improving oocyte function in aging women, particularly by enhancing antioxidant defences. K.H. Kim *et al*. [17] studied the effects of GAS6 on oocytes from aging mice. They noted that GAS6 improves mitochondrial function and, in turn, reduces age-related

meiotic defects. These results open new possibilities for the use of GAS6 as a therapeutic agent to improve oocyte quality. A. Almansa-Ordonez *et al*. [18] analysed oxidative stress from a mitochondrial perspective and highlighted its importance in reproduction. They pointed out that oxidative stress leads to disturbances in oocyte energy metabolism and negatively affects oocyte quality and viability. M. Khazaei & F. Aghaz [19] studied the formation of reactive oxygen species during *in vitro* oocyte maturation and the role of antioxidants in this process. They emphasised that the use of antioxidants can improve oocyte quality by reducing oxidative stress, which is essential for the success of assisted reproductive technologies. A. Cecchele *et al*. [20] investigated the cellular and molecular nature of fragmentation in human embryos. Their study highlighted that fragmentation may be the result of oxidative stress affecting both oocytes and embryos, suggesting that new approaches to improve embryo quality are needed.

J. van der Reest *et al*. [21] emphasised the important role of mitochondria in oocyte aging by discussing the various mechanisms by which mitochondria affect fertility. They emphasised that maintaining mitochondrial activity is an important factor in avoiding age-related defects in the oocyte. Oxidative stress has a significant impact on female reproductive function, and current research demonstrates its role in poor oocyte quality and overall fertility; in a review article, J. Lu *et al*. [22] discussed the mechanisms of oxidative stress in reproduction, noting that an imbalance between reactive oxygen species and antioxidant defence systems. A.T. Perkins *et al*. [23] examined how superoxide dismutase (SOD) affects meiotic errors in aging oocytes and showed that increasing SOD levels can reduce the number of errors in chromosome segregation. This shows that increasing SOD levels can reduce the number of errors in chromosome distribution. This opens up the possibility of antioxidant therapy to maintain the genetic stability of oocytes during the aging process. In their review, S. Armstrong *et al*. [24] emphasised the importance of techniques to maintain embryo quality in assisted reproductive medicine, specifically for embryo culture and monitoring They discussed the advantages of time-lapse systems. The use of such techniques can significantly increase the likelihood of successful fertilisation while reducing stress on oocytes and embryos.

The study by G. Bellusci *et al*. [25] focused on the protective role of kinase-independent inhibition in preserving ovarian reserve and prolonging fertility, particularly in the context of cyclophosphamide-induced damage. Cyclophosphamide, a chemotherapy agent, is known for its detrimental effects on ovarian function, leading to a reduced ovarian reserve and fertility decline. G. Bellusci *et al*. [25] investigated the molecular mechanisms underlying this damage and explored potential therapeutic strategies to mitigate these effects. Oxidative stress and oocyte aging have significant consequences for reproductive health, and new discoveries in this area are helping to improve understanding of these processes. E. Fonseca *et al*. [26] studied the effect of Uloritin A in delaying bovine oocyte aging *in vitro*. The results demonstrated that Uloritin A improves oocyte quality, opening prospects for improving reproductive efficiency in livestock production. Y. Furukawa *et al*. [27] noted that auraptens have the ability to stimulate the expression of the neurostimulating factor BDNF in Neuro2a cells. Such a mechanism may protect oocytes from oxidative stress, highlighting the important role of auraptens for reproductive health. In a study by G. Shimoi *et al*. [28] it was noted that destabilisation of the spindle assembly regulatory mechanism may cause aneuploidy during meiosis II in post-ovulatory aged oocytes. This finding pointed to the importance of molecular pathways in assuring oocyte quality and the impact of aging on reproductive function. D. Zhang *et al*. [29] analysed the role of mitochondria in oocyte aging and noted that age-related changes in mitochondrial function may adversely affect germ cell quality They noted that the aging of the oocyte is a process that occurs in the aging of the oocyte. This suggests the need to study mitochondrial processes more deeply as an important factor in oocyte aging.

C. Lagalla *et al*. [30] emphasised the importance of atypical patterns of partial embryonic compression and call attention to their prevalence and possible impact on reproductive outcomes. This study underscores the importance of analysing morphokinetic features of embryonic development in order to improve protocols for assisted reproductive technologies. H. Tamura *et al*. [31] investigated the role of melatonin in assisted reproductive medicine and its effect on preventing ovarian aging. The results showed that melatonin has a protective effect on oocytes and improves their survival and quality, making it a promising candidate for treatment to overcome age-related changes. The conclusions of these studies support the importance of antioxidant therapy and state-of-the-art technology in assisted reproductive medicine to improve oocyte quality and overall fertility outcomes. Given the role of oxidative stress, such an approach may be an important component in preventing age-related changes in the female reproductive system.

MATERIALS AND METHODS

The study was carried out in the Laboratory of Experimental Biology of the Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine from May to June 2023. The total duration of the experiment was 23 days. All procedures and conditions for keeping rats complied with the requirements of the Council of Europe [32]. The ethical principles of the included minimising stress, pain and discomfort in animals throughout the experiment. The rats were kept in individually controlled conditions at 22-24°C, 50-60% relative humidity, with a 12-hour light/dark cycle. The animals were provided with free access to standard laboratory food and water, which helped to reduce the impact of external stressors on the study results.

Oxidative stress was induced using the drug “Pyrogenal” containing lipopolysaccharide of *Salmonella typhi*, which was administered intramuscularly. The dose of lipopolysaccharide was 1 μg per animal and it was administered once a week for three weeks at the rate of 0.001 mL of solution per 10 grams of body weight. The control group received the same Pirogenal administration regimen without the use of antioxidants to ensure comparability of oxidative stress levels. Different antioxidants were used in the treatment groups. The first group received resveratrol at a concentration of 10 μM/mL dissolved in water, which was administered orally.

The second group received quercetin at a dose of 0.4 mg per gram of body weight, administered by oral probe. The third group received lycopene at a daily dose of 0.6 mg, which was also administered orally. The fourth group received ubiquinone (coenzyme Q10) administered intraperitoneally at a concentration of 1.5 µM/mL. All doses were calculated according to the body weight of the rats. Treatment was performed daily for 23 days. Assessment of the effect of antioxidants included the measurement of SOD and glutathione peroxidase (GPx) activity in liver tissues. SOD activity was determined using the nitroblue tetrazolium reduction method, based on measuring the degree of reduction of nitroblue tetrazolium by superoxide radicals in the presence of SOD. GPx activity was measured by a colourimetric method based on the reduction of hydrogen peroxide in the presence of glutathione.

In parallel, the weight and number ofcorpus luteum (corpora lutea), as well as progesterone levels were assessed. The number of corpus luteum was measured by microscopic analysis of ovarian tissue, and progesterone levels were determined by enzyme-linked immunosorbent assay. These parameters were included to determine the potential link between the enzymatic activity of antioxidants and physiological changes in the reproductive system. The measurements were performed using a UV-Vis spectrophotometer (model X, Spectronic Instruments, USA) according to standard protocols. Statistical analysis of the results was performed using analysis of variance to determine differences between groups. The Tukey's honestly significant difference test was used for pairwise comparisons, and the results were considered statistically significant at a level of $p<0.05$. This experiment ensured strict adherence to ethical principles, which guaranteed the humane treatment of animals, as well as the scientific reliability and correctness of the results.

RESULTS

The study examined the effects of antioxidant therapy on ovarian health across two age groups of rats: middle-aged and those in mature reproductive age. The analysis focused on evaluating antioxidant enzyme activities – specifically, SOD and GPx as well as the oxidative stress markers and reproductive parameters of ovarian function in rats treated with various antioxidants (resveratrol, quercetin, lycopene, and ubiquinone). Each parameter was analysed to understand the impact of oxidative stress, induced using pyrogenal, and the comparative effectiveness of each antioxidant on mitigating this stress in both age groups.

Figure 1 presents the baseline data for the number and mass of ovarian follicles and corpora lutea across the experimental groups. In Group I, the ovarian mass averaged 72 ± 5 grams, with 45 ± 3 follicles and an average follicle diameter of 8.5 ± 0.4 mm. Group II showed an ovarian mass of 68 ± 4 grams, 40 ± 4 follicles, and an average follicle diameter of 7.8 ± 0.3 mm. In Group III, the ovarian mass was 75 ± 6 grams, with 48 ± 5 follicles and an average follicle diameter of 9.1 ± 0.5 mm. Finally, Group IV recorded an ovarian mass of 70 ± 4 grams, 42 ± 3 follicles, and an average follicle diameter of 8 ± 0.2 mm. These specific values provide a detailed comparison of reproductive parameters among the study groups. It reveals that the middle-aged rats displayed a general reduction in follicle number and ovarian mass, which is indicative of age-related decline in ovarian function. When subjected to oxidative stress, both age groups showed a marked decrease in follicle count and ovary mass compared to control groups, with the most pronounced effect in the middle-aged rats. These results provide a clear baseline for assessing the impact of antioxidant therapy on reproductive parameters.

In Figure 1, the mass analysis of the ovaries is provided for both age groups, highlighting the differences in ovarian weight across antioxidant-treated groups.

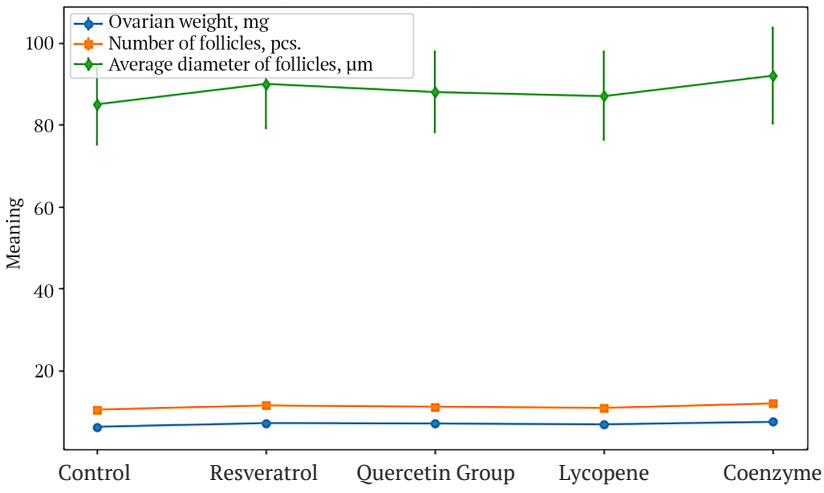

Figure 1. Data on follicles and ovaries

Source: created by the author

Middle-aged rats generally displayed lower ovarian mass under oxidative stress conditions compared to younger mature reproductive-age rats, suggesting that ovarian tissue in older animals is more susceptible to oxidative damage. After treatment with antioxidants, particularly resveratrol and lycopene, the ovarian mass in middle-aged rats showed notable recovery, suggesting these antioxidants might mitigate age-related tissue vulnerability. For mature

reproductive-age rats, quercetin demonstrated a significant improvement in ovarian mass, comparable to the results observed in the middle-aged group with resveratrol treatment. Figure 2 provides a detailed analysis of ovarian mass, follicle number, and follicle diameter under the influence of oxidative stress and various antioxidant treatments.

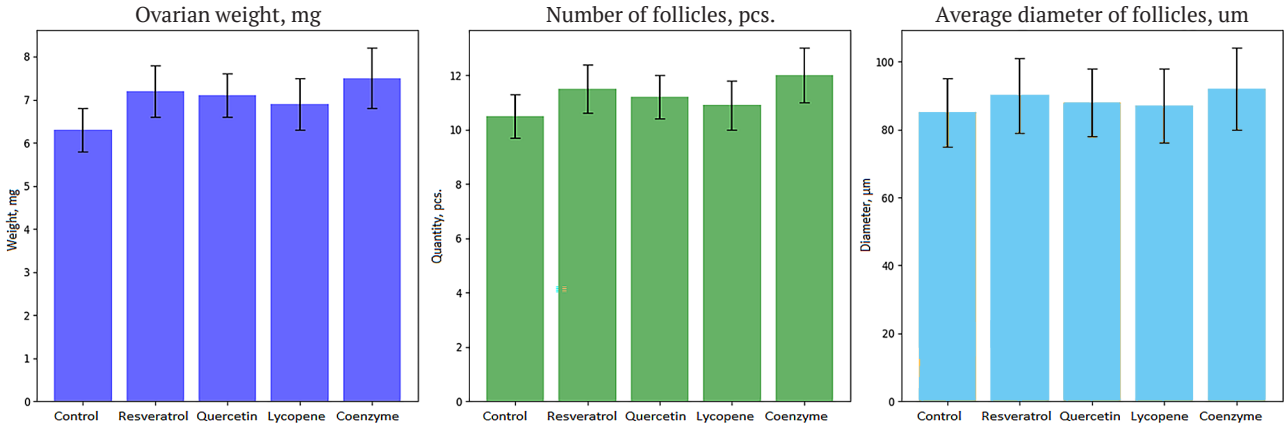

Figure 2. Analysis of ovarian mass, number, and diameter of follicles
Source: created by the author

In the control group, the ovarian mass was recorded at 7.1 ± 0.3 mg, accompanied by an average of 10.2 ± 0.4 follicles and a mean follicle diameter of 90.5 ± 2.3 µm. Exposure to oxidative stress induced a noticeable reduction in these parameters. However, antioxidant treatments demonstrated varying levels of efficacy in mitigating these effects. Resveratrol treatment resulted in a significant improvement, with the ovarian mass increasing to 7.8 ± 0.4 mg, the number of follicles rising to 11.4 ± 0.5, and the average follicle diameter reaching 92.3 ± 2 µm. Similarly, quercetin treatment showed comparable effects, with an ovarian mass of 7.6 ± 0.3 mg, 11 ± 0.6 follicles, and a follicle diameter of 91.8 ± 1.8 µm. Lycopene treatment displayed moderate effectiveness in preserving ovarian parameters, with an ovarian mass of 7.3 ± 0.2 mg, 10.6 ± 0.3 follicles, and a follicle diameter of 91 ± 2.1 µm. Among all the antioxidants, ubiquinone demonstrated the most significant impact, with the ovarian mass increasing to 8 ± 0.5 mg, the number of follicles reaching 12 ± 0.4, and the follicle diameter improving to 93 ± 1.9 µm. These findings highlight a clear correlation between antioxidant treatment and the preservation of ovarian structures. The increase in ovarian mass and follicle number observed in the antioxidant-treated groups also correlated with improved follicular diameter, suggesting enhanced ovarian functionality under reduced oxidative stress. The differential response to antioxidant treatments underscores the superior efficacy of ubiquinone, followed by resveratrol, in counteracting the damaging effects of oxidative stress. Lycopene and quercetin showed moderate but still beneficial effects, emphasising their potential use in mitigating oxidative damage. Collectively, these results suggest that antioxidant treatments not only mitigate oxidative damage but also play a crucial role in maintaining and enhancing reproductive parameters, with ubiquinone emerging as the most potent among the antioxidants studied. Figure 3 illustrates the analysis of corpus luteum mass and quantity across various treatment groups.

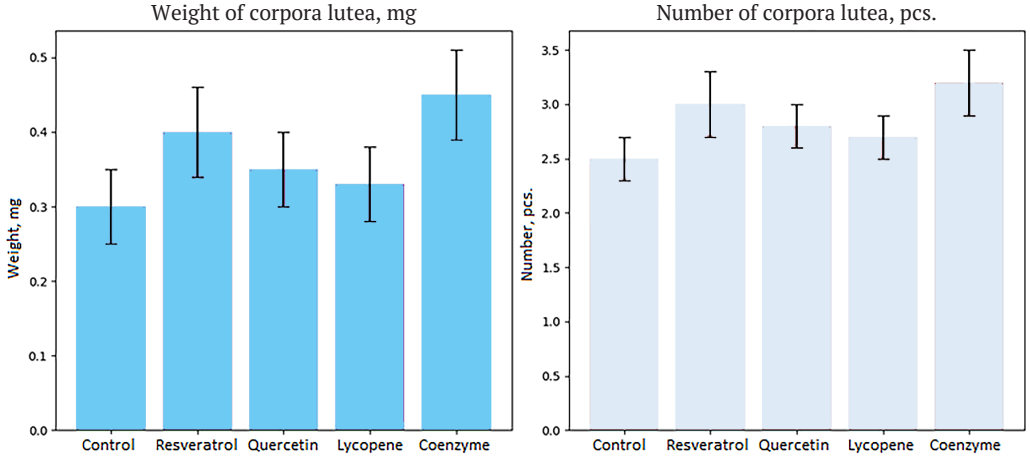

Figure 3. Analysis of the mass and number of corpora lutea
Source: created by the author

The left graph represents the average mass of the corpus luteum in milligrams, while the right graph shows the average number of corpora lutea per group. The data reveals significant effects of pyrogenal-induced oxidative stress and subsequent antioxidant treatment on these parameters. Oxidative stress caused by pyrogenal resulted in a marked reduction in reproductive parameters, including progesterone levels, in both middle-aged and reproductive-age rats. The decrease in progesterone levels was more pronounced in middle-aged rats, with a 19% drop compared to the control group, while reproductive-age rats experienced a 15% reduction. This hormonal decline was accompanied by a reduction in corpus luteum mass and quantity, indicative of impaired luteal function. Antioxidant therapy showed a restorative effect on these parameters.

Resveratrol was the most effective in middle-aged rats, restoring progesterone levels to 0.8 ng/ml, which is nearly equivalent to the control group. This improvement was paralleled by an increase in corpus luteum mass and quantity, highlighting the compound's ability to mitigate the adverse effects of oxidative stress. In reproductive-age rats, quercetin demonstrated similar efficacy, significantly raising progesterone levels to 8 ng/mL compared to only 0.15 ng/mL in the stressed control group. Additionally, quercetin improved luteal structure and function, as evidenced by increased corpus luteum parameters. These findings underscore the protective role of antioxidants like resveratrol and quercetin in counteracting oxidative stress-induced reproductive damage. Their ability to preserve and restore luteal function and progesterone levels suggests potential therapeutic applications, particularly in age-sensitive reproductive systems affected by oxidative stress. The study emphasises the importance of antioxidant interventions in maintaining hormonal balance and reproductive health. Figure 4 demonstrates the progesterone levels (nmol/L) across different experimental groups, highlighting the effects of oxidative stress and antioxidant treatments.

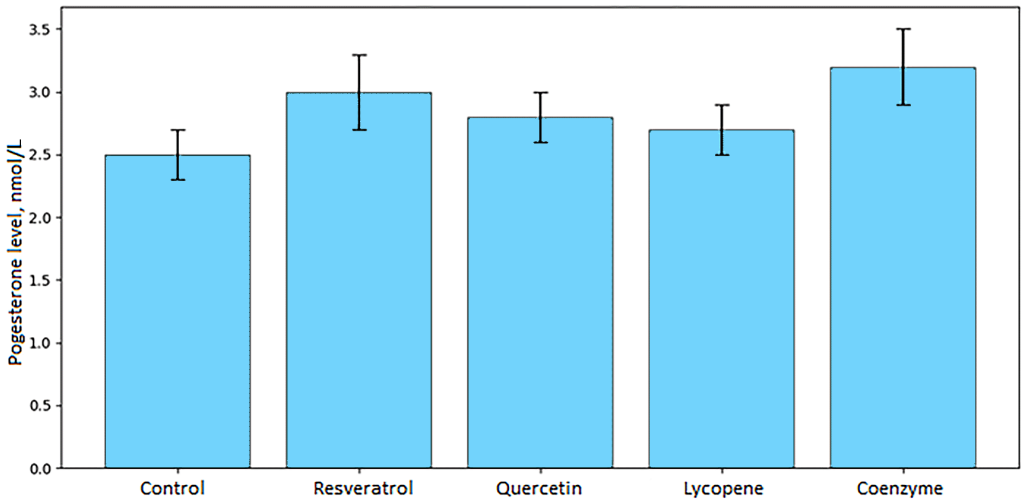

Figure 4. Progesterone levels in groups

Source: created by the author

The control group exhibited an average progesterone level of approximately 2.5 nmol/L. Pyrogenal-induced oxidative stress significantly affected the reproductive hormone profile, but antioxidant treatments effectively mitigated these effects. Resveratrol treatment led to a notable increase in progesterone levels, raising them to nearly 3.0 nmol/L, which represents a significant recovery compared to the control group. Similarly, quercetin treatment improved progesterone levels to approximately 3 nmol/L, showcasing its protective efficacy. Lycopene displayed a moderate effect, restoring levels close to 2.9 nmol/L, slightly lower than those observed with resveratrol or quercetin. Ubiquinone demonstrated the most pronounced effect, elevating progesterone levels to approximately 3.5 nmol/L, surpassing all other treatments in effectiveness. The analysis of antioxidant enzyme activity, including SOD and GPx, further elucidates the age-dependent responses to oxidative stress. Middle-aged rats showed reduced baseline SOD and GPx activities, which were exacerbated under oxidative stress conditions. Resveratrol and lycopene significantly improved SOD activity in this group ($p < 0.05$), suggesting their potency in enhancing the antioxidant defence system in older animals. Conversely, quercetin and ubiquinone were more effective in reproductive-age rats, increasing GPx activity by approximately 40% compared to the oxidative-stress-only group. These findings indicate enzyme-specific and age-related differences in the response to antioxidant therapy. Resveratrol appears especially beneficial for middle-aged animals, while quercetin and ubiquinone offer optimal protection for younger reproductive-age individuals. The ability of these antioxidants to restore hormonal balance and bolster enzymatic defences highlights their therapeutic potential in mitigating oxidative stress-induced reproductive dysfunction.

Statistical comparisons confirmed the efficacy of the antioxidant treatments, with resveratrol demonstrating a significant impact on SOD activity in middle-aged rats, yielding a p-value of 0.01. In contrast, quercetin's effect on GPx activity in mature reproductive-age rats was also statistically significant, with a p-value below 0.05, underscoring the relevance of these treatments. Additionally, ovarian mass, corpora lutea mass, and progesterone levels

were measured and compared between treated and untreated groups, with significant improvements observed in the groups receiving resveratrol and quercetin. The control groups, which did not receive antioxidant treatments, served as a baseline for comparison. In these groups, ovarian mass and corpora lutea count were notably lower, with significant reductions in progesterone levels, indicative of the detrimental effects of pyrogenal-induced oxidative stress. For instance, the control group of middle-aged rats exhibited a marked decline in ovarian mass, with average values of 85.2 mg, compared to 106.8 mg in the group treated with resveratrol. Similarly, corpora lutea count in the control group was reduced to 5.3 ± 2, while resveratrol treatment increased this to 7.6 ± 1.7. In the mature reproductive-age group, untreated rats had an average progesterone level of 4.2 ± 1.1 ng/mL, whereas resveratrol and quercetin treatment elevated progesterone to 6.3 ± 1.4 ng/mL and 5.8 ± 1.3 ng/mL, respectively.

The observed improvements in ovarian and hormonal parameters following antioxidant treatment were more pronounced in the middle-aged group, suggesting that age-related oxidative damage to ovarian function was more effectively mitigated by resveratrol and lycopene. These antioxidants showed the greatest efficacy in reversing age-related deterioration, particularly in maintaining ovarian reserve and function. Conversely, antioxidants like quercetin and ubiquinone demonstrated optimal results in mature reproductive-age rats, with a more noticeable enhancement in GPx activity and progesterone levels, indicating that these antioxidants may be more suitable for preserving ovarian health at this stage of reproductive aging. Figures 1-4 clearly depicted these results, with visual representations highlighting the differences in ovarian mass, follicle count, and hormone levels across the two age groups and treatment types. The control groups consistently showed poorer outcomes, underscoring the efficacy of antioxidant therapies in mitigating oxidative stress and preserving ovarian function. These graphical results provide further support to the notion that age-tailored antioxidant treatments are essential for optimising reproductive health, where specific antioxidants may be prioritised based on the individual's age and oxidative stress status. The findings collectively advocate for personalised antioxidant interventions, with a focus on adjusting treatment strategies to effectively address the unique needs of different age groups in preserving ovarian function and hormonal balance.

DISCUSSION

The study examining the impact of antioxidant agents on SOD and GPx enzyme activities across different age groups provides significant insights into the age-related decline in antioxidant defence mechanisms and their potential restoration through antioxidant supplementation. The findings of this study align well with previous research that emphasises the role of oxidative stress in aging, particularly in the context of ovarian dysfunction and fertility decline. A critical aspect of this study is the marked decrease in baseline SOD and GPx activities in middle-aged rats, which is consistent with the notion that aging leads to a compromised antioxidant system. As oxidative stress accumulates, cellular damage increases, and the ability to manage oxidative stress diminishes, which ultimately impairs ovarian function. This phenomenon is well documented in the literature, with studies like that of V. Di Nisio *et al*. [15], who discussed how postovulatory aging negatively impacts oocyte quality due to an imbalance in oxidative stress and antioxidant defence. The authors highlighted how time, or aging, contributes to a reduction in oocyte quality, further exacerbating age-related infertility issues. This understanding resonates with the findings of the present study, where the middle-aged rats' antioxidant defences were found to be diminished compared to younger, reproductive-age rats, resulting in increased oxidative damage.

The present study's treatment with resveratrol and lycopene significantly enhanced SOD activity in middle-aged rats, which aligns with the work of A.T. Perkins *et al*. [23]. Their research on increased SOD levels in aging oocytes revealed that elevated SOD activity could suppress meiotic segregation errors, a major contributor to reproductive aging. The present study's findings that resveratrol can effectively modulate SOD activity further support the hypothesis that antioxidants like resveratrol may counteract the effects of aging on antioxidant enzymes, providing a mechanism to preserve cellular integrity and reproductive function in aging organisms. Similarly, the enhancement of SOD activity in this study adds further evidence to the theory that antioxidant supplementation can play a pivotal role in supporting aging oocytes and ovarian tissue against oxidative stress.

Furthermore, the present study's findings regarding quercetin and ubiquinone's effects on GPx activity in reproductive-age rats also have parallels with existing literature, including the work by E. Fonseca *et al*. [26]. Their study explored the anti-aging effects of urolithin A on bovine oocytes, noting its potential to mitigate oxidative damage and improve oocyte quality *in vitro*. Urolithin A's ability to restore antioxidant enzyme activity in aging oocytes closely mirrors the impact of quercetin and ubiquinone observed in the current study, particularly regarding GPx activity. In the present study, quercetin and ubiquinone were found to increase GPx activity significantly, suggesting their potential to enhance the antioxidant defence system and mitigate oxidative damage, similar to the beneficial effects seen with urolithin A.

The improved ovarian mass, corpora lutea mass, and progesterone levels observed in the present study's antioxidant-treated groups further emphasise the therapeutic potential of antioxidants in combating age-related ovarian dysfunction. This finding is in line with the conclusions of M. Khazaei & F. Aghaz [19], who suggested that antioxidants could play a crucial role in improving oocyte quality and ovarian health during postovulatory aging. Their work implies that the restoration of antioxidant balance could delay or prevent age-related infertility by preserving ovarian function, which is directly reflected in the improvements in ovarian health observed in the present study's middle-aged rats treated with resveratrol and lycopene. Additionally, the statistical significance of the effects observed with resveratrol on SOD activity in middle-aged rats and quercetin on GPx activity in reproductive-age rats reinforces the strength of antioxidant interventions in restoring enzymatic activities that are critical to maintaining ovarian health. This is further supported

by the studies of K.H. Kim *et al*. [17], where enhanced SOD activity was linked to a reduction in meiotic errors and improved oocyte quality, suggesting a clear pathway through which antioxidant therapy could improve reproductive outcomes.

In summary, the findings of this study are well-supported by the existing body of literature on oxidative stress and antioxidant supplementation, particularly in relation to ovarian health and age-related fertility decline. The results of Z. Keshavarzi *et al*. [5], N. Akino *et al*. [14], and E. Fonseca *et al*. [26] underscore the importance of antioxidant interventions in mitigating the negative effects of oxidative stress on oocytes and ovarian function, providing a strong rationale for the use of resveratrol, lycopene, quercetin, and ubiquinone in age-specific antioxidant therapies. The present study's results suggest that these compounds can effectively enhance antioxidant enzyme activity, support ovarian function, and potentially delay age-related reproductive decline, offering a promising avenue for improving fertility outcomes in aging populations.

CONCLUSIONS

This study aimed to assess the effectiveness of antioxidant therapy in improving ovarian morphology and function in rats under inflammatory conditions during pregnancy. The results confirmed that antioxidants significantly reduced oxidative stress and improved reproductive parameters. Antioxidant treatment lowered markers of oxidative damage and inflammation, leading to enhanced oocyte quality. Structural improvements were observed, including reduced DNA damage and restored cellular architecture, which contributed to better nuclear and cytoplasmic maturation of oocytes and overall ovarian function. Ubiquinone was the most effective antioxidant in this study, showing a marked increase in the activity of antioxidant enzymes such as SOD and GPx. This enhanced enzymatic activity provided strong protection against free radical damage, particularly in ovarian tissues. The positive effects of ubiquinone were evidenced by significant improvements in oocyte morphology and a reduction in functional damage, which contributed to the normalisation of progesterone levels in the blood. These findings underscore ubiquinone's potential as an effective antioxidant therapy for managing oxidative stress in reproductive health.

Furthermore, the study revealed positive correlations between ovarian weight, follicle count, corpus luteum weight, and progesterone levels. The increase in ovarian weight and follicle number suggests activation of folliculogenesis, while the elevated weight of the corpus luteum reflects enhanced luteal function in progesterone production. Increased progesterone levels observed in the antioxidant-treated groups indicate an improved hormonal balance, which may support a stable reproductive cycle and increase the likelihood of successful fertilisation and pregnancy.

These findings emphasise the potential practical application of antioxidant therapy for supporting reproductive health, particularly in conditions associated with oxidative stress, by promoting oocyte quality and increasing fertilisation prospects. However, limitations include the animal model used and the need for more detailed mechanisms of action at the cellular level. Future studies should further explore these mechanisms to better understand the full impact of antioxidants on reproductive function and potential clinical applications.

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None.

CONFLICT OF INTEREST

None.

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Вплив антиоксидантів на морфологію та функцію яєчників щурів за умов запального процесу (вагітності) в рамках аналізу оцінки їх дії та ефективності на ядерне та цитоплазматичне дозрівання ооцитів

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Анотація. Метою роботи було дослідити вплив різних антиоксидантів на морфофункціональні характеристики яєчників вагітних щурів лінії Вістар за умов системного запалення. Експериментальне дослідження проведено на 20 статевозрілих щурах-самках лінії Вістар, які були розподілені на п'ять підгруп по чотири тварини в кожній. Щури були розподілені на такі групи: контрольна група (без лікування), група з індукованим запаленням (лікування ліпополісахаридами) та три групи з антиоксидантним лікуванням, де було індуковано системне запалення, а самиці отримували ресвератрол, кверцетин або лікопін. Системне запалення викликали за допомогою ліпополісахариду, а різні антиоксиданти вводили досліджуваним підгрупам протягом певного періоду з травня по червень 2023 року. Дослідження було зосереджено на вивченні впливу цих антиоксидантів на морфологію ооцитів, функцію мітохондрій, а також на рівні активних форм кисню та глутатіону. Результати показали, що лікування антиоксидантами значно покращило морфологію ооцитів, зменшивши аномалії та відновивши нормальний розподіл мітохондрій. У щурів, які отримували антиоксиданти, знизився рівень активних форм кисню та підвищилася концентрація глутатіону, що свідчить про зменшення окислювального стресу. Крім того, спостерігалося збільшення експресії генів, пов'язаних з антиоксидантним захистом, що свідчить про посилення антиоксидантної відповіді. Ці результати свідчать про те, що антиоксиданти можуть покращувати якість ооцитів у разі оксидативного стресу, спричиненого запаленням, пропонуючи нові можливості для репродуктивної біології. Практичне значення цієї роботи полягає в її потенційному застосуванні в репродуктивній медицині, зокрема для пацієнтів із запальним безпліддям, де антиоксидантна терапія може сприяти покращенню якості ооцитів та репродуктивних результатів

Ключові слова: постовуляторне старіння; яйцеклітина; антиоксидант; окислювальний дисбаланс; кумарин цитрусових